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Short Report: Low Prevalence of *Leishmania* Infection in Post-Epidemic Areas of Libo Kemkem, Ethiopia

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Abstract. In Libo Kemkem (a district of Amhara region, Ethiopia), no cases of kala-azar had ever been reported until 2005 when an outbreak occurred. Over one-third of those cases were children under 15 years of age. The aim of the present study was to determine the prevalence of *Leishmania* infection in children aged 4–15 years. A cross-sectional survey was conducted in 2009. Children participating in the survey were selected using a three-stage cluster sampling method. A total of 386 children were included in the study. The overall prevalence of *Leishmania* infection (direct agglutination test- and/or rK39 immunochromatographic test- and/or leishmanin skin test-positive subjects) in this population was 1.02% (95% confidence interval = 0–4.54), and prevalence was higher in boys and children older than 12 years. Only one case of active disease was encountered. The results suggest that the conditions responsible for the outbreak no longer reign. However, active surveillance remains necessary.

East Africa has suffered a marked increase in the number of cases of visceral leishmaniasis (VL) or kala-azar over the last two decades, probably because of a combination of demographic and climatic changes.[1,2]

The areas traditionally regarded as endemic for VL in Ethiopia lie in the northwest (bordering Sudan) and south of the country.[3,4] Libo Kemkem district (wereda) is located in the highlands of the Amhara region in northwestern Ethiopia at an altitude of 1,800–2,000 m. According to year 2007 census, population of Libo Kemkem was 198,374, and 69,388 people were children between 4 and 15 years of age.[5]

No cases of kala-azar had ever been declared in this area until 2005. Earlier, in 2004, the Amhara Regional Health Bureau reported a fivefold increase in crude mortality rates in the Libo Kemkem district, and this finding was attributed to an outbreak of drug-resistant malaria. However, in May of 2005, an outbreak of kala-azar caused by *Leishmania donovani* was determined to be the culprit.[6,7] The epidemiological background (a few cases over a 1-year period followed by an explosive increase) was consistent with the rapid emergence of the disease in a population with little pre-existing immunity.[6] By December of 2007, 2,543 patients with kala-azar had been treated by Médecins sans Frontières.[8] More than one-third of the patients were children under 15 years of age, and a fatality rate of over 3% was reported for this group.[8] The rapid spread of the disease between 2004 and 2007 suggested that transmission would not be easy to control.[6] Before the Libo Kemkem outbreak, there was no epidemiological surveillance system for leishmaniasis in Ethiopia, making it difficult to determine whether the epidemic between 2004 and 2007 was an outbreak caused by a recent introduction of the parasite or as suggested in the work by Herrero and others,[8] the parasite being endemic to the area but in low numbers.

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The aim of the present study was to determine the prevalence of *Leishmania* infection in children aged 4–15 years from Libo Kemkem 4 years after the outbreak.

A cross-sectional survey was conducted between May and July of 2009 as part of the project Visceral Leishmaniasis and Malnutrition in Amhara State, Ethiopia that was funded by the UBS-Optimus Foundation; among its specific objectives were aims to characterize nutritional, immunological, and parasitological aspects in the school-aged child population from this area. Sampling was undertaken as part of a multistaged cluster survey. The primary sampling units were randomly selected subdistricts (kebeles) of Libo Kemkem that, according to the records of Médecins sans Frontières-Greece held at the Addis Zemen Health Center, had reported at least one case of VL during the 2004–2007 epidemic. These areas were selected taking into account their size according to a recent census.[5] The secondary sampling units were randomly selected villages (gotts) in each of the selected subdistricts. The tertiary sampling units were households randomly selected from an updated census for each village. All children between 4 and 15 years of age residing in these households were tested. A total of 386 children were included in the study.

Ethical clearance was obtained from the review boards of the Instituto de Salud Carlos III, the Armauer Hansen Research Institute, and the Ethiopian National Ethical Review Committee. Parents/guardians gave written, informed consent before the enrolment of their children in the study. For children over 11 years of age, verbal assent was obtained in addition to the consent of their parents or guardians.

Each participant was clinically assessed by health professionals for any complaint of fever lasting longer than 2 weeks, weight loss, and presence of splenomegaly and lymphadenopathy to determine the presence of any active infection. All children were tested using the leishmanin skin test (LST), the rK39 immunochromatographic test (rK39-ICT, Kalazar Detect Rapid Test; InBios International Inc., Seattle, WA), and the direct agglutination test (DAT) (ITMA-DAT/VL; Institute of Tropical Medicine, Antwerp, Belgium). Sociodemographic data were recorded using pretested questionnaires. The rK39-ICT test was performed immediately after blood sampling according to the manufacturer's instructions. The DAT

test was performed on blood-impregnated filter paper using freeze-dried antigen. The screening method followed the manufacturer's protocol; titers of ≥ 1:3,200 were deemed positive. Leishmanin skin testing was performed using *L. major* antigen (Leishmanin batch 123–2; Pasteur Institute, Tehran, Iran) as previously described.[9,10]

The overall prevalence of *Leishmania* infection (DAT- and/or rK39-ICT- and/or LST-positive) in the population was then calculated. Prevalence rates were expressed in percentages with 95% confidence intervals (95% CIs). Stata v.10.1 software was used to perform all statistical analyses. Data were weighted according to selection probabilities and analyzed using the Stata v.10.1 complex samples procedures, which takes into account sample clustering.

Of 386 children, 199 (50.9%) were girls. The mean age of the participants was 8.9 years (standard deviation [SD] = 3.03); 44.76% of the children were under 8 years of age, and 33.08% of the children were between 8 and 11 years.

Only one case of active VL was found, which returned positive results for both rK39-ICT and DAT. Nine children were DAT-positive only; four of these children had suffered kala-azar previously. However, five children that previously had VL showed negative results for both rK39-ICT and DAT. None of the children returned a positive LST result. Post–kala-azar dermal leishmaniasis (PKDL) or cutaneous leishmaniasis cases were not found.

The overall prevalence of infection (DAT- and/or rk39-positive) was 1.02% (95% CI = 0–4.54). The prevalence among boys was higher (1.78%, 95% CI = 0–7.98 in boys; 0.3%, 95% CI = 0–1.31 in girls). The greatest prevalence was recorded in children older than 12 years (2.56%, 95% CI = 0–10.54) followed by those children between 8 and 11 years (0.82%, 95% CI = 0–3.53); the under 8 years subgroup showed the lowest prevalence (0.49%, 95% CI = 0–2.59). However, these prevalence values were not statistically different from one another (Table 1).

To the best of our knowledge, these data are the first *Leishmania* infection prevalence data for Libo Kemkem since the 2004–2007 epidemic. Higher prevalence rates for similar populations have, however, been reported for other regions of Ethiopia.[11,12]

The permanence of leishmanin skin test reactivity is thought to depend on latent infection and continuous exposure to the biting of *Leishmania*-carrying sand flies[11,13]; a decrease of these vectors and the establishment of a VL treatment center after the 2004–2007 outbreak could have contributed to the reduction of reservoirs, which might explain the low prevalence of active cases in the present study population.[8] The vector decrease can only be possible as a consequence of important environmental events, because it happened in a short period of time after the outbreak. This finding highlights the need for both additional entomological studies and establishment of a surveillance system to avoid other catastrophic outbreaks in the study area and neighboring places.

Some works report a natural conversion rate from positive to negative of 14.8–9.3% in other settings in Ethiopia.[14] Nevertheless, the absence of positive results is noteworthy, especially compared with previous results in the same age range (23.6%),[6] although in the latter study, rapid assessment by convenience sampling was undertaken.

The present study also used DAT and rK39-ICT for detecting infection, both of which reveal the presence of anti-*Leishmania* antibodies appearing early after infection. The combined use of these methods, which has performed well in VL diagnosis in this area,[15] yielded just a 1% prevalence for the 4- to 15-year-old age range. The prevalence of *Leishmania* infection is expected to increase with age[11]; however, the presence of only one active case in the study population and the low prevalence even among the older children suggest a low prevalence for the general population. The response to the 2004–2007 outbreak included the establishment of centers where specific treatment could be received. The clearance of patients' infections would have diminished the number of reservoirs, thus contributing to a reduction in the seroprevalence rate for the whole population.

The discordances observed could be because of the different immune responses detected by the different tests; LST measures delayed type hypersensitivity, whereas serology measures levels of anti-*Leishmania* antibodies. LST positivity appears later after infection and seems to be a sign of protective immunity against VL, whereas seropositivity is considered a marker of more recent infection and has been related to disease progression.[16,17]

The present results seem to indicate that the conditions that provoked the kala-azar epidemic in the study region no longer reign. The parasite may have been introduced by migrant agricultural laborers who, returning to their villages after completing seasonal work on the border of Sudan,[18] acted as a reservoir of the causal parasite—a hypothesis put forward at the time of the epidemic. However, the available evidence indicates that the affected population was not made up simply of migrant workers, and there is no evidence that any such migration has ever ceased. This finding suggests

TABLE 1
Prevalence of *Leishmania* infection

	Sample		Prevalence of *Leishmania* infection (rK39-ICT and/or DAT-positive)				
	*N**	Percent†	*N**	Percent†	95% CI†	OR (95% CI)	*P*
Overall	386		10	1.02	0–4.54		
Sex							
Girls	199	50.9	2	0.3	0–1.31	Reference	
Boys	187	49.1	8	1.78	0–7.98	5.94 (0.38–93.84)	0.291‡
Age (years)							
< 8	169	44.76	2	0.49	0–2.59	Reference	
8–11	132	33.08	3	0.82	0–3.53	1.675 (0.1–29.12)	
> 11	85	22.16	5	2.56	0–10.54	5.228 (0.43–63.4)	0.39‡

*Unweighted.
†Weighted.
‡Fisher.

that, as well as an increase in the size of the human reservoir, some change in the vector population must have occurred. Libo Kemkem lies at an altitude of 1,800–2,000 m, which is beyond the altitude at which phlebotomes are normally found. Changes in temperature (because of global warming) or soil type (because of land use) could have been the reason for either colonization of the Libo Kemkem area by sand fly vectors or propagation of a native population, reaching high enough levels to increase leishmaniasis transmission.[19–21] New entomological studies should be carried out to elucidate the role of sand fly vectors.

It should be noted, however, that the response to the outbreak included the establishment of centers where specific treatment could be received. The clearance of patients' infections would have led to a reduction in the seroprevalence rate.

Until 2004, no case of leishmaniasis had ever been reported in Libo Kemkem. The very low prevalence in the study population may suggest that the district may be experiencing a pre-epidemic status similar to the status seen before the outbreak. Efforts to identify areas of high prevalence and then focus control efforts in these places might be wiser than blanket control of the entire district. However, the doubts surrounding the reasons for the outbreak mean that vigilance with respect to the impact of possible climate changes should be considered; such changes might encourage new outbreaks.

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